NOVEL GENE THAT IS AMPLIFIED AND OVEREXPRESSED IN CANCER AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

This invention relates to the fields of molecular biology and neoplastic disease, and more specifically, to isolated nucleic acids, proteins, antibodies, methods and kits containing the same which are useful in genetic screening assays, and in the design of clinically beneficial chemotherapeutic agents which inhibit the aberrant cellular proliferation in tumor cells.

BACKGROUND OF THE INVENTION

Several publications are referenced in this application by author name and year of publication in parentheses in order to more fully describe the state of the art to which this invention pertains. The disclosure of each of these publications is incorporated by reference herein.

Pancreatic cancer is the fifth leading cause of death by cancer in the United States. Twenty-four thousand people die each year from this disease. The 5-year survival for pancreatic cancer patients is less than 5% and the incidence of the disease has tripled over the last 40 years. The molecular basis

underlying the pathogenesis of pancreatic
adenocarcinoma remains unknown. As a result, the
disease has an extremely poor prognosis and lacks
early diagnostic and therapeutic modalities.

5   Normal cellular proliferation is finely regulated
by the expression of growth-promoting proto-oncogenes
and growth-controlling anti-oncogenes. Mutations,
rearrangements, deletions, or amplifications that
potentiate the activities of proto-oncogenes result in
10   tumor formation. Similar events that inactivate anti-
oncogenes or tumor suppressor genes disrupt their role
in the cell as negative regulators of cell growth and
proliferation.

          Gene amplification has been implicated as a
15   common mechanism by which tumor cells acquire a
chemotherapy resistant phenotype. Some amplification
units arise at the site of the normal gene, but
disperse into the cytoplasm as double minutes (DMs).
These DMs may become reincorporated and reamplified as
20   homogeneously staining regions (HSRs) or abnormal
banding regions (ABRs) at other sites in the genome.
DMs and HSRs may be alternate forms of amplified DNA.
The DMs are not inherited stably during cell division
because of the lack of centromeres. Integration of
25   DMs into a chromosome is thought to result in the
formation of HSRs, which represent a more stable,
form of the amplified DNA which is maintained as the
cell divides. The mechanisms underlying this process
are not completely understood, but appear to be based
30   on recombination and unequal distribution of the
amplified DNA into daughter cells.

          Cytogenetic amplification has been observed in 8
of 63 primary pancreatic adenocarcinomas analyzed for

the presence of DMs. The expression of epidermal
growth factor, epidermal growth factor receptor,
transforming growth factor, erbB-2, erbB-3, and c-met
are elevated in pancreatic cancer (Barton et al.,

5   1991; Korc et al., 1992; Lemoine et al., 1992; Prat et
al., 1991).

       Using restriction landmark genomic scanning
(RLGS), Miwa et al (1996) identified a locus at
chromosome 19q13.1-q13.2 including the AKT2 gene which

10   was amplified in 20 % of pancreatic cancer. Over
expression of the AKT2 gene was further shown to be
associated with the malignant phenotype of a subset
of human ductal pancreatic cancers (Cheng et al.,
1996).

15   No well-defined differentiation pathway has been
shown in pancreatic adenocarcinomas, and the biology
of this tumor type is generally poorly understood.
The present inventors have appreciated a need for the
isolation of essential components involved in the

20   regulation of differentiation and proliferation of
pancreatic tumor cells. Molecular elucidation of
these components will provide novel targets for the
development of antiproliferative and diagnostic agents
for cancer treatment and diagnosis.

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SUMMARY OF THE INVENTION
       This invention provides novel, biological
molecules useful for identification, detection, and/or
molecular characterization of components involved in

30   the regulation of cellular differentiation and
tumorigenesis. According to one aspect of the
invention, an isolated nucleic acid molecule is
provided which includes an isolated open reading frame

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encoding a phosphoprotein of a size between about 60
to 70 kilodaltons. The encoded protein, referred to
herein as PD2, comprises a tripartite domain structure
including a nuclear transport signal, a helix-loop-

5   helix domain, a nucleotide binding site, and several
putative phosphorylation sites.

In a preferred embodiment of the invention, an
isolated nucleic acid molecule is provided that
includes a DNA encoding a human PD2 protein. In a

10   particularly preferred embodiment, the human PD2
protein has an amino acid sequence the same as SEQ ID
NO:2. An exemplary PD2 nucleic acid molecule of the
invention comprises SEQ ID NO:1.

According to another aspect of the present

15   invention, an isolated nucleic acid molecule is
provided, which has a sequence selected from the group
consisting of: (1) SEQ ID NO:1; (2) a sequence
specifically hybridizing with preselected portions or
all of the complementary strand of SEQ ID NO:1; (3) a

20   sequence comprising preselected portions of SEQ ID
NO:1, (4) a sequence encoding part or all of a
polypeptide having amino acid SEQ ID NO:2. Such
partial sequences are useful as probes to identify and
isolate homologues of the PD2 gene of the invention.

25   Accordingly, isolated nucleic acid sequences encoding
natural allelic variants of the nucleic acids of SEQ
ID NO:1 are also contemplated to be within the scope
of the present invention. The term natural allelic
variants will be defined hereinbelow.

30   According to another aspect of the present
invention, isolated human PD2 protein is provided.
PD2 is a phosphoprotein with a deduced molecular
weight of between about 60 kDa and 70 kDa. PD2

comprises a tripartite domain structure including a
nuclear transport signal, a helix-loop-helix domain, a
cyclic AMP or related nucleotide binding site, and
several putative phosphorylation sites. The

5   expression of this PD2 protein correlates with the
deregulated growth of highly undifferentiated
pancreatic adenocarcinomas.

In a preferred embodiment of the invention, the
protein is of human origin, and has the amino acid

10   sequence of SEQ ID NO:2. In a further embodiment the
protein may be encoded by natural allelic variants of
SEQ ID NO:1. Inasmuch as certain amino acid
variations may be present in human PD2 protein encoded
by natural allelic variants, such proteins are also

15   contemplated to be within the scope of the invention.

According to another aspect of the present
invention, antibodies immunologically specific for the
human PD2 protein described hereinabove are provided.

Host cells comprising the PD2 encoding nucleic

20   acids of the invention are also contemplated to be
within the scope of the present invention. Such host
cells include but are not limited to bacterial cells,
mammalian cells, insect cells, fungal cells, and plant
cells. The PD2- encoding nucleic acids may be

25   conveniently cloned into a plasmid or retroviral
vector for introduction into host cells. Such cells
are useful in screening methods to identify compounds
which regulate and/or inhibit PD2 expression.
Compounds so identified may have therapeutic value in

30   the treatment of pancreatic cancer.

The present invention also encompasses transgenic
mice expressing the PD2 encoding nucleic acids of the
invention. The PD2 encoding DNA may be altered to

include any of the following, mutations, alterations, deletions, insertions. In another embodiment, PD2 knockout mice may be generated to assess the contribution of the PD2 gene to growth and

5   development.

This invention also provides methods for genetic screening and diagnostic evaluation of patients at risk for, or currently suffering from, cancer of the pancreas. The hybridization specificity of the

10   nucleic acids of the invention may be used for differential evaluation of patients presenting with phenotypic characteristics common to pancreatic cancer. The nucleic acid molecules of the invention can be used as diagnostic hybridization probes or as

15   primers for diagnostic PCR analysis for PD2 or mutations thereof. Additionally, antisense molecules which may be useful in the regulation of PD2 expression are provided herein. Other methods encompassed by the present invention include

20   immunodetection methods for assessing biological samples for the presence of PD2 proteins.

In another aspect of the present invention, kits are provided for practicing the methods set forth above. An exemplary kit for screening tumor samples

25   for PD2 expression includes for example, suitable primers for PCR amplification of target PD2 sequences. Exemplary primers include those having the sequence of SEQ ID NOS: 12 and 13. A kit in accordance with the invention may also contain vials, buffers, a target

30   PD2 sequence as a positive control and a protocol sheet. Another exemplary kit may employ immunological methodology. Kits of this type include immobilized PD2 protein and antibodies immunologically specific

for PD2. Such kits may be used in for immunological
assessment of biopsy specimens for identification
and/or quantification of PD2 in pancreatic tissues.

       The term "isolated nucleic acid" is sometimes
5   used with reference to nucleic acids of the invention.
This term, when applied to DNA, refers to a DNA
molecule that is separated from sequences with which
it is immediately contiguous (in the 5' and 3'
directions) in the naturally occurring genome of the
10   organism from which it originates. For example, the
"isolated nucleic acid" may comprise a DNA or cDNA
molecule inserted into a vector, such as a plasmid or
virus vector, or integrated into the genomic DNA of a
prokaryote or eukaryote.

15   When used with reference to RNA molecules of the
invention, the term "isolated nucleic acid" primarily
refers to an RNA molecule encoded by an isolated DNA
molecule as defined above. Alternatively, the term
may refer to an RNA molecule that has been
20   sufficiently separated from RNA molecules with which
it would be associated in its natural state (i.e., in
cells or tissues), such that it exists in a
substantially pure form.

       The terms "isolated protein" or "isolated and
25   purified protein" are sometimes used herein to refer
to a protein produced by expression of an isolated
nucleic acid molecule of the invention.
Alternatively, these terms may refer to a protein
which has been sufficiently separated from other
30   proteins with which it would naturally be associated,
so as to exist in substantially pure form.

       The term "substantially pure" refers to a
preparation comprising at least 50-60% by weight of

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the compound of interest (e.g., nucleic acid, oligonucleotide, protein, etc.). More preferably, the preparation comprises at least 75% by weight, and most preferably 90-99% by weight, the compound of interest.

5   Purity is measured by methods appropriate for the compound of interest (e.g. chromatographic methods, agarose or polyacrylamide gel electrophoresis, HPLC analysis, and the like).

With respect to antibodies of the invention, the

10   term "immunologically specific" refers to antibodies that bind to one or more epitopes of a protein of interest (e.g., PD2), but which do not substantially recognize and bind other molecules in a sample containing a mixed population of antigenic biological

15   molecules.

With respect to nucleic acids and oligonucleotides, the term "specifically hybridizing" refers to the association between two single-stranded nucleotide molecules of sufficiently complementary

20   sequence to permit such hybridization under pre-determined conditions generally used in the art (sometimes termed "substantially complementary"). When used in reference to a double stranded nucleic acid, this term is intended to signify that the double

25   stranded nucleic acid has been subjected to denaturing conditions, as is well known to those of skill in the art. In particular, the term refers to hybridization of an oligonucleotide with a substantially complementary sequence contained within a single-

30   stranded DNA or RNA molecule of the invention, to the substantial exclusion of hybridization of the oligonucleotide with single-stranded nucleic acids of non-complementary sequence.

The nucleic acids, proteins, and antibodies, of the present invention may be used to advantage diagnostic reagents and tools for assessing the malignant potential of pancreatic adenocarcinomas. They may also be used as targets for the development of novel chemotherapeutic agents that regulate differentiation and/or inhibit aberrant cellular proliferation in tumor cells. The transgenic mice of the invention provide a means to assess the function of PD2 in vivo.

The human PD2 molecules described are above may also be used as research tools and will facilitate the elucidation of the genetic and protein interactions involved in the regulation of cell division, differentiation, and neoplastic transformation. Methods and kits employing such molecules are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show autoradiographs of Northern blot analysis of RNA isolated from differentiated and undifferentiated pancreatic cell lines. Total RNA (20 μg; lanes 1 and 2) or poly(A+) RNA (lanes 3 and 4) was separated by gel electrophoresis and transferred to a nitrocellulose membrane. Lanes 1 and 3 contained RNA from the differentiated pancreatic cell line HPAF/CD-11. Lanes 2 and 4 contained RNA from the highly undifferentiated pancreatic cell line Panc 1. Figure 1A shows the hybridization of a ^{32}P-labelled PD2 cDNA probe. PD2 is expressed at 30-fold higher levels in Panc 1 cells as compared to HPAF/CD-11 cells. Figure 1B shows an autoradiogram of the same blot hybridized with a ^{32}P-

labelled β-actin probe confirming equivalent loading
of RNA per lane.

Figure 2 depicts the nucleotide (SEQ ID NO:1) and
deduced amino acid (SEQ ID NO:2) sequence of PD2 cDNA.
The nucleotide sequence is numbered in the left-hand
margin. The deduced amino acid sequence is numbered
in the right-hand margin. The polyadenylation signal
is underlined.

Figure 3 depicts the *in vitro* transcription/
translation product of PD2 cDNA. RNA transcripts were
generated from pBluescript using T7 RNA polymerase and
translated in rabbit reticulocyte lysate in the
presence of ^{35}S methionine. The protein products were
separated by SDS-polyacrylamide gel electrophoresis
and visualized by autoradiography. The DNA constructs
used to generate the transcription/translation
products or the negative controls were as follows:
lane 1, PD2 cDNA inserted in pBluescript in the sense
orientation; lane 2, PD2 cDNA inserted in pBluescript
in the antisense orientation; lane 3, pBluescript
linearized with HindIII; Lane 4, control rabbit
reticulocyte lysate containing no exogenous
transcripts; Lane 5, construct containing cDNA for
PD-1 in the sense orientation.

Figures 4A and 4B schematically represent the PD2
primary amino acid sequence, from amino acid 1 through
531. Figure 4A reveals the relative positions of
three putative protein motifs: a helix-loop-helix
domain, a nuclear localization domain, and an Arg-rich
RNA binding / cAMP binding domain, indicated by boxes.

Figure 4B uses boxes to depict the regions of sequence
identity between various proteins (listed in Table 1)
and the deduced amino acid sequence of PD2 protein.

5   Figure 5 shows the alignment of the helix-loop-
helix domain of the deduced PD2 amino acid sequence
with the helix-loop-helix domain of the *Drosophila
Hairy* protein (20). The top sequence labeled "A."
represents amino acid residues 30 - 87 of the
10   *Drosophila Hairy* protein, and the bottom sequence
labled "B." represents amino acid residues 15 - 77 of
the PD2 protein. Note that the amino acid residues
within the "loop" regions are not directly shown or
listed in the alignment. Boxed residues indicate
15   identity between the two sequences. Asterisks (*)
indicate identity in the PD2 sequence with other
members of the helix-loop-helix family of proteins
(35).

20   Figure 6 shows an alignment of PD2 residues with
consensus cAMP binding domains of the bacterial
catabolite activator protein, and the regulatory
subunit of the eukaryotic cAMP-dependent protein
kinase. Row A shows residues 198 - 213 of the RIß
25   isoform of the regulatory type I subunit of cAMP
dependent protein kinase (16). Row B shows residues
322-337 of the RIß isoform of the regulatory type I
subunit of cAMP dependent protein kinase (16). Row C
shows residues 69 - 88 of the *E. coli* catabolite gene
30   activator protein (16). Row D shows residues 322 -
341 of the deduced PD2 sequence (Figure 2). Identity
among the amino acid sequences is indicated with boxes
around the identical residues.

Figure 7 depicts immunoblotting analysis of
cytoplasmic and nuclear protein fractions from Panc 1
and HPAF/CD11 cells. Equal amounts of cytoplasmic and
nuclear protein lysates from each cell line were
5   loaded onto an SDS-PAGE gel and electroblotted onto
nitrocellulose. The PD2p2 polyclonal antibody reacted
with a band of about 70 kd and 3 smaller fragments of
approximately 40-45 kd in the nuclear extracts
isolated from both Panc1 and HPAF/CD-11 cells.
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Figures 8A and 8B are autoradiographs depicting
Northern blot analyses of total RNA (20 μg/lane) from
a panel of pancreatic carcinoma cell lines. Total RNA
was fractionated by electrophoresis on 1.2%
15   agarose/formaldehyde gel and transferred to a
nitrocellulose membrane. Pancreatic carcinoma cell
lines analyzed were as follows: Colo 357 (lane 1),
Fa-2C (lane 2), Panc 89 (lane 3), Panc 1 (lane 4),
Capan 2 (lane 5), HS 766T (lane 6), SW 979 (lane 7),
20   T3M4 (lane 8), HPAF (lane 9), BxPC 3 (lane 10), AsPC 1
(lane 11), QGP-1 (lane 12), MiaPaCa (lane 13) and HGC-
25 (lane 14). Figure 8A shows the results obtained
following hybridization with a ^{32}P-labelled PD2 cDNA
probe. Figure 8B shows hybridization results obtained
25   using a ^{32}P-labelled ß-actin cDNA probe confirming
equivalent loading of RNA per lane.

Figures 9A and 9B are autoradiographs depicting
Northern blot analysis showing expression levels of
30   PD2 mRNA in normal and tumor cell lines and tissues.
Total RNA (20 μg/lane) was fractionated by
electrophoresis on a 1.2% agarose/formaldehyde gel and
transferred to a nitrocellulose membrane. The cell

lines and tissues examined were: pancreatic tumor cell
lines Panc 1 (lane 4) and HPAF (lane 3); breast
carcinoma cell lines BT 20 (lane 1) and CAMA-1 (lane
2); colon carcinoma cell lines LS 180 (lane 6) and

5   Colo 320 (lane 7); normal human pancreas (lane 5);
tail portion of pancreas (lane 8); pancreatic tumors
(lanes 9, 10 and 11); normal human foreskin
fibroblasts (lane 12); and human B lymphocyte cell
line NALM (lane 13). Figure 9A shows hybridization

10   results obtained using a ^{32}P-labelled PD2 cDNA probe.
Figure 9B depicts the results obtained using a ^{32}P-
labelled ß-actin cDNA probe, to confirm equivalent
loading of RNA per lane.

15   Figure 10 is an autoradiograph of Southern blot
analysis of genomic DNA (10μg/lane) from the Panc 1
and HPAF/CD11 pancreatic tumor cell lines. After
restriction endonuclease digestion, the DNA was
electrophoretically separated on a 0.8% agarose gel

20   and transferred to a nitrocellulose membrane by
standard methods. Lanes 1, 2 and 3 contained Panc 1
DNA digested with EcoRI, HindIII and BamHI,
respectively. Lanes 4, 5, and 6 contained HPAF/CD11
DNA digested with EcoRI, HindIII and BamHI,

25   respectively. The blot was probed with a ^{32}P-labelled
PD2 cDNA probe, revealing the 30-fold amplification of
the PD2 gene in Panc 1 cells as compared to HPAF/CD11.

Figure 11 is an autoradiograph showing results

30   from Southern blot analysis of genomic DNA (10μg/lane)
from a panel of tumor cell lines. After restriction
endonuclease digestion with EcoRI, the DNA was
electrophoretically separated on a 0.8% agarose gel.

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Cell lines analyzed included: pancreatic cell lines
HPAF/CD11 (lane 1), Panc 1 (lane 2), Colo 357 (lane
3), SW 979 (lane 4), Capan-1 (lane 5), T3M4 (lane 6),
and Hs 766T (lane 7); breast cancer cell lines: CAMA-
5   1 (lane 8), MCF-7 (lane 9), Sk-BR (lane 10). Colon
carcinoma cell lines: WiDr (lane 11), Colo 320 (lane
12) and LS 180 (lane 13). The blot was probed with ^{32}P
labelled PD2 cDNA.

10   Figure 12 is an autoradiograph illustrating that
the PD2 gene is amplified in human pancreatic biopsies
and tumor cell lines. Purified DNA (10 μg) was
digested with PstI (P) and BglII (B), fractionated on
8% agarose and transferred to a nylon membrane. The
15   blot was probed with a ^{32}P-labelled probe specific for
PD2 (upper panel). The same blot was stripped and
rehybridized to a control probe to confirm equal
loading in each lane. Tumor (T) and normal tissue
adjacent to tumor (C). DNA from patient cases 1-4 are
20   shown in lanes 1-16. Tumor DNA isolated from cases 5
and 6 are shown in lanes 17-20. Pancl DNA is shown in
lanes 21 and 22.

Figure 13 is an an ethidium bromide stained gel
25   showing the results of RT-PCR on normal human and
adult pancreatic tissues. RT-PCR products from normal
fetal and adult pancreas were fractionated on a 1.5%
agarose gel. Lanes 1-3, adult pancreas; lanes 4-6,
fetal pancreas; M, 100 base pair ladder.

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Figure 14 depicts immunoblot analysis of the
epitope-tagged PD2 protein. After transfection of the
epitope-tagged PD2 cDNA construct in the pancreatic

tumor cells, clones were isolated for G418 resistance.
Lysates for three clones were analyzed. Protein bands
were resolved by SDS-PAGE, transferred to
nitrocellulose and probed with M2 monoclonal antibody
5   which specifically recognizes the flag epitope. C,
cytoplasmic lysate; N, nuclear lysate; M, molecular
weight marker.

        Figure 15 shows an autoradiogram of a SDS-PAGE
10   gel run under reducing conditions showing
phosphorylation of PD2 protein in the presence of ^{32}P-
orthophosphate. APR, vector alone; PD2F, APR vector
with PD2 Flag sequences; C, cytoplasmic; N, nuclear.

15   Figure 16 is an autoradiogram showing the results
of Northern analysis of transfected NIH3T3 cells.
Total cellular RNA (10μg) purified from human control
and transfected cells was fractionated on a 1%
agarose/formaldehyde gel, blotted, and hybridized with
20   a cDNA probe to PD2.

        Figure 17 is a graph showing the growth curve for
PD2 transfected NIH3T3 cell lines.

25   Figure 18 is a graph showing the kinetics of
tumor growth of PD2(sense)NIH3T3 cells inoculated into
BALB/c nu/nu mice.

DETAILED DESCRIPTION OF THE INVENTION.
30   Adenocarcinomas of the pancreatic ducts make up
over 95% of pancreatic nonendocrine tumors, although
the duct system accounts for a minor proportion of the
normal gland. Accurate staging of pancreatic cancer

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is required to evaluate treatment modalities such as
surgical resection, radiotherapy and chemotherapy. A
universally satisfactory staging system has not been
devised, although several staging systems have been
5   introduced for clinical practice (reviewed in
Eskelinen and Lipponen, 1992). The grading of
pancreatic adenocarcinomas utilizes generally accepted
principles of glandular differentiation, nuclear size
and mitotic activity (Kloppel et al., 1985), but
10   subjectivity in assessment and heterogeneity are
common. Accurate histological classification of these
tumors accordingly, has prognostic relevance and
should aid in the selection of appropriate therapy.

Well-differentiated tumors (grade 1) contain duct
15   like structures with polarized cells. Moderately
differentiated tumors (grade 2) contain less
differentiated duct-like and tubular glands. Poorly
differentiated tumors (grade 3) contain pleomorphic
structures, poorly-differentiated glands, minimal
20   mucus production and large nuclei. The most
differentiated tumors grow as tubular structures with
a common luminal space, while less differentiated
tumors show a loss of cell polarity resulting in
secretion into both luminal and interstitial space
25   (Kern et al., 1987). The tumor growth rate of poorly-
differentiated tumors is twice that for well
differentiated tumors. Median survival times are
correlated with the tumor grade: patients with poorly-
differentiated tumors survived for a shorter time than
30   did patients with well-differentiated tumors (Kloppel
et al., 1985; Eskelinen and Lipponen, 1992).

In an effort to identify differentially expressed
or overexpressed genes that play a role in maintaining

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distinct morphological differentiation features
exhibited by pancreatic adenocarcinoma cells, a cDNA
library from a poorly differentiated human pancreatic
tumor cell line, Panc 1 (1,2), was screened with

5   single stranded cDNA probes generated from mRNA
prepared from Panc 1 and a well-differentiated human
pancreatic tumor cell line, HPAF/CD11 (3). One cDNA
clone (PD-2) detected an mRNA expressed at levels 30-
fold higher in Panc 1 cells, as compared to HPAF/CD-11

10 cells. A similar amplification was observed in the
gene copy number by Southern analysis. The present
invention provides the nucleotide sequence, deduced
amino acid sequence, and chromosomal location of this
previously undescribed cDNA. The availability of

15 these sequences enables the practice of genetic
screening assays for classifying particular pancreatic
tumors as well as providing novel targets for the
development of clinically relevant chemotherapeutic
agents.

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I.   Preparation of Human PD2-Encoding Nucleic Acid
       Molecules, PD2 Proteins, and Antibodies Thereto

25   A.   Nucleic Acid Molecules
            Nucleic acid molecules encoding the human
PD2 proteins of the invention may be prepared by two
general methods: (1) synthesis from appropriate
nucleotide triphosphates, or (2) isolation from

30 biological sources. Both methods utilize protocols
well known in the art. The availability of nucleotide
sequence information, such as a cDNA having the
sequence of SEQ ID NO:1 enables preparation of an

isolated nucleic acid molecule of the invention by
oligonucleotide synthesis. Synthetic oligonucleotides
may be prepared by the phosphoramidite method employed
in the Applied Biosystems 38A DNA Synthesizer or

5   similar devices. The resultant construct may be
purified according to methods known in the art, such
as high performance liquid chromatography (HPLC).
Long, double-stranded polynucleotides, such as a DNA
molecule of the present invention, must be synthesized

10   in stages, due to the size limitations inherent in
current oligonucleotide synthetic methods. Thus, for
example, a 1.9 kb double-stranded molecule may be
synthesized as several smaller segments of appropriate
complementarity. Complementary segments thus produced

15   may be annealed such that each segment possesses
appropriate cohesive termini for attachment of an
adjacent segment. Adjacent segments may be ligated by
annealing cohesive termini in the presence of DNA
ligase to construct an entire 1.9 kb double-stranded

20   molecule. A synthetic DNA molecule so constructed may
then be cloned and amplified in an appropriate vector.
        Nucleic acid sequences encoding the human PD2
protein may be isolated from appropriate biological
sources using methods known in the art. In a

25   preferred embodiment, a cDNA clone is isolated from a
cDNA expression library of human origin. In an
alternative embodiment, utilizing the sequence
information provided by the cDNA sequence, human
genomic clones encoding PD2 proteins may be isolated.

30   Suitable probes for this purpose are derived from
sequences within the PD2 cDNA and include the
following sequences:

5' AGTGACAAGAGTGGCAGTGG 3'   (SEQ ID NO: 3)

5' GAGGACAGAGGACAGGCCCA 3'   (SEQ ID NO: 4)   :

5' CACTCGGCCCAGGAGGATGG 3'   (SEQ ID NO: 5)

5' GACAGTGACTGAGTCCCAGG 3'   (SEQ ID NO: 6)

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      Such probes may be between 15 and 40 nucleotides
in length. For probes longer than those shown above,
the additional contiguous nucleotides are provided
within SEQ ID NO:1.

10   Additionally, cDNA or genomic clones having
homology with human PD2 may be isolated from other
species using oligonucleotide probes corresponding to
predetermined sequences within the human PD2 encoding
nucleic acids.

15   In accordance with the present invention, nucleic
acids having the appropriate level of sequence
homology with the protein coding region of SEQ ID NO:1
may be identified by using hybridization and washing
conditions of appropriate stringency. For example,

20 hybridizations may be performed, according to the
method of Sambrook et al., Molecular Cloning, Cold
Spring Harbor Laboratory (1989), using a hybridization
solution comprising: 5X SSC, 5X Denhardt's reagent,
1.0% SDS, 100 μg/ml denatured, fragmented salmon sperm

25 DNA, 0.05% sodium pyrophosphate and up to 50%
formamide. Hybridization is carried out at 37-42°C
for at least six hours. Following hybridization,
filters are washed as follows: (1) 5 minutes at room
temperature in 2X SSC and 1% SDS; (2) 15 minutes at

30 room temperature in 2X SSC and 0.1% SDS; (3) 30
minutes-1 hour at 37°C in 1X SSC and 1% SDS; (4) 2
hours at 42-65°C in 1X SSC and 1% SDS, changing the
solution every 30 minutes.

One common formula for calculating the
stringency conditions required to achieve
hybridization between nucleic acid molecules of a
specified sequence homology (Sambrook et al., 1989) is
5   as follows:

$$T_m = 81.5°C + 16.6 Log\ [Na+] + 0.41(\%\ G+C) - 0.63\ (\%\ formamide) - 600/\#bp\ in\ duplex$$

As an illustration of the above formula, using
10   [Na+] = [0.368] and 50% formamide, with GC content of
42% and an average probe size of 200 bases, the T_m is
57°C. The T_m of a DNA duplex decreases by 1 - 1.5°C
with every 1% decrease in homology. Thus, targets
with greater than about 75% sequence identity would be
15   observed using a hybridization temperature of 42°C.
Nucleic acids of the present invention may be
maintained as DNA in any convenient cloning vector.
In a preferred embodiment, clones are maintained in a
plasmid cloning/expression vector, such as pBluescript
20   (Stratagene, La Jolla, CA), which is propagated in a
suitable *E. coli* host cell.
PD2-encoding nucleic acid molecules of the
invention include cDNA, genomic DNA, RNA, and
fragments thereof which may be single- or double-
25   stranded. Thus, this invention provides
oligonucleotides having sequences capable of
hybridizing with at least one sequence of a nucleic
acid molecule of the present invention, such as
selected segments of the cDNA having SEQ ID NO:1. As
30   mentioned previously, such oligonucleotides are useful
as probes for detecting or isolating PD2 genes.
Antisense nucleic acid molecules may be targeted
to translation initiation sites and/or splice sites to

inhibit the expression of the PD2 gene or production
of the PD2 protein of the invention. Such antisense
molecules are typically between 15 and 30 nucleotides
in length and often span the translational start site
of PD2 encoding mRNA molecules. Suitable antisense
molecules for controlling the expression of PD2 are as
follows:
5' CTGGATGGTGGGCGCCATA 3'   (SEQ ID NO:7)
5' CCTGGTCCCGCTTTCGTTT 3'   (SEQ ID NO:8)
5' CTAAGGCGGACCCTGGTTT 3'   (SEQ ID NO:9)

     Alternatively, antisense constructs may be
generated which contain the entire PD2 cDNA in reverse
orientation. Such antisense constructs are
exemplified herein.
     It will be appreciated by persons skilled in the
art that variants (e.g., allelic variants) of PD2
sequences exist in the human population, and must be
taken into account when designing and/or utilizing
oligonucleotides of the invention. Accordingly, it is
within the scope of the present invention to encompass
such variants, with respect to the PD2 sequences
disclosed herein or the oligonucleotides targeted to
specific locations on the respective genes or RNA
transcripts. Accordingly, the term "natural allelic
variants" is used herein to refer to various specific
nucleotide sequences of the invention and variants
thereof that would occur in a human population. The
usage of different wobble codons and genetic
polymorphisms which give rise to conservative or
neutral amino acid substitutions in the encoded
protein are examples of such variants. Additionally,
the term "substantially complementary" refers to

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oligonucleotide sequences that may not be perfectly matched to a target sequence, but such mismatches do not materially affect the ability of the oligonucleotide to hybridize with its target sequence

5   under the conditions described.

        B. **Proteins**

        Full-length human PD2 protein of the present invention may be prepared in a variety of ways,

10   according to known methods. The protein may be purified from appropriate sources, e.g., transformed bacterial or animal cultured cells or tissues, by immunoaffinity purification. However, this is not a preferred method due to the low amount of protein

15   likely to be present in a given cell type at any time. The availability of nucleic acid molecules encoding PD2 protein enables production of the protein using *in vitro* expression methods known in the art. For example, a cDNA or gene may be cloned into an

20   appropriate *in vitro* transcription vector, such as pSP64 or pSP65 for *in vitro* transcription, followed by cell-free translation in a suitable cell-free translation system, such as wheat germ or rabbit reticulocyte lysates. *In vitro* transcription and

25   translation systems are commercially available, e.g., from Promega Biotech, Madison, Wisconsin or Gibco-BRL, Gaithersburg, Maryland.

        Alternatively, according to a preferred embodiment, larger quantities of PD2 protein may be

30   produced by expression in a suitable prokaryotic or eukaryotic system. For example, part or all of a DNA molecule, such as a cDNA having SEQ ID NO:1 may be inserted into a plasmid vector adapted for expression

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in a bacterial cell, such as *E. coli*. Such vectors comprise the regulatory elements necessary for expression of the DNA in the host cell positioned in such a manner as to permit expression of the DNA in

5   the host cell. Such regulatory elements required for expression include promoter sequences, transcription initiation sequences and, optionally, enhancer sequences.

The human PD2 protein produced by gene

10   expression in a recombinant procaryotic or eukaryotic system may be purified according to methods known in the art. In a preferred embodiment, a commercially available expression/secretion system can be used, whereby the recombinant protein is expressed and

15   thereafter secreted from the host cell, and readily purified from the surrounding medium. If expression/secretion vectors are not used, an alternative approach involves purifying the recombinant protein by affinity separation, such as by

20   immunological interaction with antibodies that bind specifically to the recombinant protein or nickel columns for isolation of recombinant proteins tagged with 6-8 histidine residues at their N-terminus or C-terminus. Alternative tags may comprise the FLAG

25   epitope or the hemagglutinin epitope. Such methods are commonly used by skilled practitioners.

The human PD2 protein of the invention, prepared by the aforementioned methods, may be analyzed according to standard procedures. For example, such

30   protein may be subjected to amino acid sequence analysis, according to known methods.

The present invention also provides antibodies capable of immunospecifically binding to proteins of

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the invention. Polyclonal antibodies directed toward
human PD2 protein may be prepared according to
standard methods. In a preferred embodiment,
monoclonal antibodies are prepared, which react

5   immunospecifically with the various epitopes of the
PD2 protein described herein. Monoclonal antibodies
may be prepared according to general methods of Köhler
and Milstein, following standard protocols.
Polyclonal or monoclonal antibodies that

10   immunospecifically interact with PD2 protein can be
utilized for identifying and purifying such protein.
For example, antibodies may be utilized for affinity
separation of proteins with which they
immunospecifically interact. Antibodies may also be

15   used to immunoprecipitate proteins from a sample
containing a mixture of proteins and other biological
molecules. Other uses of anti-PD2 antibodies are
described below.

20   II.   Uses of PD2-Encoding Nucleic Acids, PD2 Proteins
and Antibodies Thereto
Cellular signalling molecules have received a
great deal of attention as potential prognostic
indicators of neoplastic disease and as therapeutic

25   agents to be used for a variety of purposes in cancer
chemotherapy. The PD2 protein of the invention is
intimately involved in the regulation of
differentiation and neoplastic growth. The
biochemical and molecular interactions of the PD2 gene

30   and protein involved in the genesis and maintenance of
the transformed state and in the maintenance of an
undifferentiated state provide novel targets for the
development of chemotherapeutic reagents that may be

used to block the growth of tumor cells and/or promote
differentiation.

Additionally, PD2 nucleic acids, proteins and
antibodies thereto, according to this invention, may
be used as research tools to identify other proteins
that are involved in differentiation and
transformation processes.

A.   PD2-Encoding Nucleic Acids

PD2-encoding nucleic acids may be used for a
variety of purposes in accordance with the present
invention.   PD2-encoding DNA, RNA, or fragments
thereof may be used as probes to detect the presence
of and/or expression of genes encoding PD2 proteins.
Methods in which PD2-encoding nucleic acids may be
utilized as probes for such assays include, but are
not limited to: (1) *in situ* hybridization; (2)
Southern hybridization (3) northern hybridization; and
(4) assorted amplification reactions such as
polymerase chain reactions (PCR).

The PD2-encoding nucleic acids of the invention
may also be utilized as probes to identify related
genes from other animal species.   As is well known in
the art, hybridization stringencies may be adjusted to
allow hybridization of nucleic acid probes with
complementary sequences of varying degrees of
homology.

Thus, PD2-encoding nucleic acids may be used to
advantage to identify and characterize other genes of
varying degrees of relation to the PD2 genes of the
invention thereby enabling further characterization of
the aberrant cell growth associated with pancreatic
adenocarcinomas.   Additionally, the nucleic acids of

the invention may be used to identify genes encoding
proteins that interact with PD2 proteins (e.g., by the
"interaction trap" technique), which should further
accelerate identification of the components involved
5   in regulation of cellular proliferation.

Nucleic acid molecules, or fragments thereof,
encoding PD2 genes may also be utilized to control the
production of PD2 proteins, thereby regulating the
amount of protein available to participate in the
10   maintenance of deregulated cell growth. As mentioned
above, antisense oligonucleotides corresponding to
essential processing sites in PD2-encoding mRNA
molecules may be utilized to inhibit PD2 protein
production in targeted cells. Alterations in the
15   physiological amount of PD2 proteins may dramatically
affect the activity of other protein factors involved
in the regulation of cell division.

The PD2 nucleic acids of the invention may be
introduced into host cells. In a preferred
20   embodiment, mammalian cell lines are provided with
comprise a PD2-encoding nucleic acid or a variant
thereof. Host cells contemplated for use include, but
are not limited to NIH3T3, CHO, HELA, yeast, bacteria,
insect and plant cells. The PD2 encoding nucleic
25   acids may be operably linked to appropriate regulatory
expression elements suitable for the particular host
cell to be utilized. Methods for introducing nucleic
acids into host cells are well known in the art. Such
methods include, but are not limited to, transfection,
30   transformation, calcium phosphate precipitation,
electroporation and lipofection.

The host cells described above may be used as
screening tools to identify compounds which modulate

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PD2 activity. Modulation of PD2 activity may be
assessed by measuring alterations in PD2
phosphorylation in the presence of the test compound.
The morphology of PD2 expressing cells may also be

5   altered in the presence of the test compound.
Finally, test compounds will be assessed for the
induction of certain pancreatic differentiation
markers, such as MUC-1 and carbonic anhydrase.

         The availability of PD2 encoding nucleic acids

10   enables the production of strains of laboratory mice
carrying part or all of the PD2 gene or mutated
sequences thereof, in single or amplified copies.
Such mice may provide an in vivo model for cancer, and
may be particularly useful in studying pancreatic

15   cancer. Alternatively, the human PD2 nucleic acid
sequence information provided herein enables the
cloning of the murine homolog for use in the
production of knockout mice in which the endogenous
gene encoding PD2 has been specifically inactivated.

20   Methods of introducing transgenes and knockouts in
laboratory mice are known to those of skill in the
art. Three common methods include: 1. integration of
retroviral vectors encoding the foreign gene of
interest into an early embryo; 2. injection of DNA

25   into the pronucleus of a newly fertilized egg; and 3.
the incorporation of genetically manipulated embryonic
stem cells into an early embryo. Production of the
transgenic and knockout mice described above will
facilitate the molecular elucidation of the role PD2

30   proteins play in differentiation and tumorigenesis.
         The alterations to the PD2 gene envisioned
herein include modifications, deletions, and
substitutions. Modifications and deletions render the

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naturally occurring gene nonfunctional, producing a
"knock out" animal. Substitutions of the naturally
occurring gene for a gene from a second species
results in an animal which produces an PD2 gene from

5   the second species. Substitution of the naturally
occurring gene for a gene having a mutation results in
an animal with a mutated PD2 protein. A transgenic
mouse carrying the human PD2 gene is generated by
direct replacement of the mouse PD2 gene with the

10   human gene. These transgenic animals are valuable for
use in vivo assays for elucidation of other medical
disorders associated with cellular activities
modulated by PD2 genes. A transgenic animal carrying
a "knock out" of a PD2 encoding nucleic acid is useful

15   for the establishment of a nonhuman model for
pancreatic cancer involving PD2 regulation.

       As a means to define the role that PD2 plays in
mammalian systems, mice can be generated that cannot
make PD2 proteins because of a targeted mutational

20   disruption of a PD2 gene.

       The term "animal" is used herein to include all
vertebrate animals, except humans. It also includes
an individual animal in all stages of development,
including embryonic and fetal stages. A "transgenic

25   animal" is any animal containing one or more cells
bearing genetic information altered or received,
directly or indirectly, by deliberate genetic
manipulation at the subcellular level, such as by
targeted recombination or microinjection or infection

30   with recombinant virus. The term "transgenic animal"
is not meant to encompass classical cross-breeding or
in vitro fertilization, but rather is meant to
encompass animals in which one or more cells are

altered by or receive a recombinant DNA molecule.
This molecule may be specifically targeted to defined
genetic locus, be randomly integrated within a
chromosome, or it may be extrachromosomally

5   replicating DNA. The term "germ cell line transgenic
animal" refers to a transgenic animal in which the
genetic alteration or genetic information was
introduced into a germ line cell, thereby conferring
the ability to transfer the genetic information to

10   offspring. If such offspring in fact, possess some or
all of that alteration or genetic information, then
they, too, are transgenic animals.

The alteration or genetic information may be
foreign to the species of animal to which the

15   recipient belongs, or foreign only to the particular
individual recipient, or may be genetic information
already possessed by the recipient. In the last case,
the altered or introduced gene may be expressed
differently than the native gene.

20   The altered PD2 gene generally should not fully
encode the same PD2 protein native to the host animal
and its expression product should be altered to a
minor or great degree, or absent altogether. However,
it is conceivable that a more modestly modified PD2

25   gene will fall within the compass of the present
invention if it is a specific alteration.

The DNA used for altering a target gene may be
obtained by a wide variety of techniques that include,
but are not limited to, isolation from genomic

30   sources, preparation of cDNAs from isolated mRNA
templates, direct synthesis, or a combination thereof.

A preferred type of target cell for transgene
introduction is the embryonal stem cell (ES). ES

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cells may be obtained from pre-implantation embryos cultured in vitro. Transgenes can be efficiently introduced into the ES cells by standard techniques such as DNA transfection or by retrovirus-mediated

5   transduction. The resultant transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The introduced ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal.

10   One approach to the problem of determining the contributions of individual genes and their expression products is to use isolated PD2 genes to selectively inactivate the wild-type gene in totipotent ES cells (such as those described above) and then generate

15   transgenic mice. The use of gene-targeted ES cells in the generation of gene-targeted transgenic mice is known in the art.

Techniques are available to inactivate or alter any genetic region to a mutation desired by using

20   targeted homologous recombination to insert specific changes into chromosomal alleles. However, in comparison with homologous extrachromosomal recombination, which occurs at a frequency approaching 100%, homologous plasmid-chromosome recombination was

25   originally reported to only be detected at frequencies between 10^{-6} and 10^{-3}. Nonhomologous plasmid-chromosome interactions are more frequent occurring at levels 10^{5}-fold to 10^{2}-fold greater than comparable homologous insertion.

30   To overcome this low proportion of targeted recombination in murine ES cells, various strategies have been developed to detect or select rare homologous recombinants. One approach for detecting

homologous alteration events uses the polymerase chain
reaction (PCR) to screen pools of transformant cells
for homologous insertion, followed by screening of
individual clones. Alternatively, a positive genetic
5   selection approach has been developed in which a
marker gene is constructed which will only be active
if homologous insertion occurs, allowing these
recombinants to be selected directly. One of the most
powerful approaches developed for selecting homologous
10   recombinants is the positive-negative selection (PNS)
method developed for genes for which no direct
selection of the alteration exists. The PNS method is
more efficient for targeting genes which are not
expressed at high levels because the marker gene has
15   its own promoter. Non-homologous recombinants are
selected against by using the Herpes Simplex virus
thymidine kinase (HSV-TK) gene and selecting against
its nonhomologous insertion with effective herpes
drugs such as gancyclovir (GANC) or (1-(2-deoxy-2-
20   fluoro-B-D arabinofluranosyl)-5-iodouracil, (FIAU).
By this counter selection, the number of homologous
recombinants in the surviving transformants can be
increased.

As used herein, a "targeted gene" or "knock-out"
25   is a DNA sequence introduced into the germline or a
non-human animal by way of human intervention,
including but not limited to, the methods described
herein. The targeted genes of the invention include
DNA sequences which are designed to specifically alter
30   cognate endogenous alleles.

Methods of use for the transgenic mice of the
invention are also provided herein. Knockout mice of
the invention can be injected with tumor cells or

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treated with carcinogens to generate carcinomas. Such
mice provide a biological system for assessing the
role played by a PD2 gene of the invention.
Accordingly, therapeutic agents which inhibit the
5   action of PD2 proteins may be screened in studies
using PD2 knock out mice.

As described above, PD2-encoding nucleic acids
are also used to advantage to produce large quantities
of substantially pure PD2 proteins, or selected
10   portions thereof.

B.   PD2 Protein and Antibodies

Purified PD2 protein, or fragments thereof,
may be used to produce polyclonal or monoclonal
15   antibodies which also may serve as sensitive detection
reagents for the presence and accumulation of PD2
protein (or complexes containing PD2 protein) in
mammalian cells. Recombinant techniques enable
expression of fusion proteins containing part or all
20   of PD2 protein. The full length protein or fragments
of the protein may be used to advantage to generate an
array of monoclonal antibodies specific for various
epitopes of PD2 protein, thereby providing even
greater sensitivity for detection of PD2 protein in
25   cells.

Polyclonal or monoclonal antibodies
immunologically specific for PD2 protein may be used
in a variety of assays designed to detect and
quantitate the protein. Such assays include, but are
30   not limited to: (1) flow cytometric analysis; (2)
immunochemical detection/localization of PD2 protein
in tumor cells or cells in various stages of
differentiation; and (3) immunoblot analysis (e.g.,

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dot blot, Western blot) of extracts from various
cells. Additionally, as described above, anti-PD2
antibodies can be used for purification of PD2 protein
and any associated subunits (e.g., affinity column

5   purification, immunoprecipitation).

From the foregoing discussion, it can be seen
that PD2-encoding nucleic acids, PD2 expressing
vectors, PD2 protein and anti-PD2 antibodies of the
invention can be used to detect PD2 gene expression

10   and alter PD2 protein accumulation for purposes of
assessing the genetic and protein interactions
involved in the control of differentiation and
transformation pathways.

15   C. Methods of use for the Compositions of the
invention and Kits for preforming the disclosed
methods.

From the foregoing discussion, it can be seen
that PD2-encoding nucleic acids, PD2-expressing

20   vectors, PD2 proteins and anti-PD2 antibodies of the
invention can be used to detect PD2 gene expression
and alter PD2 protein accumulation for purposes of
assessing the genetic and protein interactions
involved in malignant transformation of pancreatic

25   cells.

Exemplary approaches for detecting PD2 nucleic
acid or polypeptides/proteins include:

a) comparing the sequence of nucleic acid in the
sample with the PD2 nucleic acid sequence to determine

30   whether the sample from the patient contains
mutations;or

b) determining the presence, in a sample from a
patient, of the polypeptide encoded by the PD2 gene

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and, if present, determining whether the polypeptide
is full length, and/or is mutated, and/or is expressed
at the normal level; or

c) using DNA restriction mapping to compare the
restriction pattern produced when a restriction enzyme
cuts a sample of nucleic acid from the patient with
the restriction pattern obtained from normal PD2 gene
or from known mutations thereof; or,

d) using a specific binding member capable of
binding to a PD2 nucleic acid sequence (either normal
sequence or known mutated sequence), the specific
binding member comprising nucleic acid hybridizable
with the PD2 sequence, or substances comprising an
antibody domain with specificity for a native or
mutated PD2 nucleic acid sequence or the polypeptide
encoded by it, the specific binding member being
labelled so that binding of the specific binding
member to its binding partner is detectable; or,

e) using PCR involving one or more primers based
on normal or mutated PD2 gene sequence to screen for
normal or mutant PD2 gene in a sample from a patient.

A "specific binding pair" comprises a specific
binding member (sbm) and a binding partner (bp) which
have a particular specificity for each other and which
in normal conditions bind to each other in preference
to other molecules. Examples of specific binding pairs
are antigens and antibodies, ligands and receptors and
complementary nucleotide sequences. The skilled person
is aware of many other examples and they do not need
to be listed here. Further, the term "specific binding
pair" is also applicable where either or both of the
specific binding member and the binding partner
comprise a part of a large molecule. In embodiments in

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which the specific binding pair are nucleic acid
sequences, they will be of a length to hybridize to
each other under conditions of the assay, preferably
greater than 10 nucleotides long, more preferably
greater than 15 or 20 nucleotides long.

In most embodiments for screening for cancer
susceptibility alleles, the PD2 nucleic acid in the
sample will initially be amplified, e.g. using PCR, to
increase the amount of the analyte as compared to
other sequences present in the sample. This allows
the target sequences to be detected with a high degree
of sensitivity if they are present in the sample. This
initial step may be avoided by using highly sensitive
array techniques that are becoming increasingly
important in the art.

The identification of the PD2 gene and its
association with cancer paves the way for aspects of
the present invention to provide the use of materials
and methods, such as are disclosed and discussed
above, for establishing the presence or absence in a
test sample of a variant form of the gene, in
particular an allele or variant specifically
associated with cancer, especially pancreatic cancer.
This may be for diagnosing a predisposition of an
individual to cancer. It may be for diagnosing cancer
of a patient with the disease as being associated with
the gene.

In still further embodiments, the present
invention concerns immunodetection methods for
binding, purifying, removing, quantifying or otherwise
generally detecting biological components. The encoded
proteins or peptides of the present invention may be
employed to detect antibodies having reactivity

therewith, or, alternatively, antibodies prepared in accordance with the present invention, may be employed to detect the encoded proteins or peptides.

In general, the immunobinding methods include obtaining a sample suspected of containing a protein, peptide or antibody, and contacting the sample with an antibody or protein or peptide in accordance with the present invention, as the case may be, under conditions effective to allow the formation of immunocomplexes.

The immunobinding methods include methods for detecting or quantifying the amount of a reactive component in a sample, which methods require the detection or quantitation of any immune complexes formed during the binding process. Here, one would obtain a sample suspected of containing a PD2 gene encoded protein, peptide or a corresponding antibody, and contact the sample with an antibody or encoded protein or peptide, as the case may be, and then detect or quantify the amount of immune complexes formed under the specific conditions.

In terms of antigen detection, the biological sample analyzed may be any sample that is suspected of containing the PD2 antigen, such as a pancreas or lymph node tissue section or specimen, a homogenized tissue extract, an isolated cell, a cell membrane preparation, separated or purified forms of any of the above protein-containing compositions, or even any biological fluid that comes into contact with pancreatic tissues, including blood and lymphatic fluid.

Contacting the chosen biological sample with the protein, peptide or antibody under conditions

effective and for a period of time sufficient to allow
the formation of immune complexes (primary immune
complexes) is generally a matter of simply adding the
composition to the sample and incubating the mixture

5   for a period of time long enough for the antibodies to
form immune complexes with, i.e., to bind to, any
antigens present. After this time, the sample-antibody
composition, such as a tissue section, ELISA plate,
dot blot or Western blot, will generally be washed to

10   remove any non-specifically bound antibody species,
allowing only those antibodies specifically bound
within the primary immune complexes to be detected.

In general, the detection of immunocomplex
formation is well known in the art and may be achieved

15   through the application of numerous approaches. These
methods are generally based upon the detection of a
label or marker, such as any radioactive, fluorescent,
biological or enzymatic tags or labels of standard use
in the art. U.S. Patents concerning the use of such

20   labels include U.S. Pat. Nos. 3,817,837; 3,850,752;
3,939,350; 3,996,345; 4,277,437; 4,275,149 and
4,366,241, each incorporated herein by reference. Of
course, one may find additional advantages through the
use of a secondary binding ligand such as a second

25   antibody or a biotin/avidin ligand binding
arrangement, as is known in the art.

The immunodetection methods of the present
invention have evident utility in the diagnosis of
pancreatic cancer. Here, a biological or clinical

30   sample suspected of containing either the encoded
protein or peptide or corresponding antibody is used.
However, these embodiments also have applications to
non-clinical samples, such as in the titering of

antigen or antibody samples, in the selection of
hybridomas, and the like.

In the clinical diagnosis or monitoring of
patients with pancreatic cancer, the detection of PD2

5   antigen, or an increase in the levels of such an
antigen, in comparison to the levels in a
corresponding biological sample from a normal subject
is indicative of a patient with pancreatic cancer. The
basis for such diagnostic methods lies, in part, with

10   the finding that the PD2 nucleic acid identified in
the present invention is overexpressed in pancreatic
cancer tissue samples (see Examples below). By
extension, it may be inferred that this nucleic
produces elevated levels of encoded PD2 proteins which

15   may also be used as pancreatic cancer markers.

As mentioned previously, cell lines expressing
the PD2-encoding nucleic acids or variants thereof may
be used in screening methods to identify agents which
modulate PD2 function.

20   In one broad aspect, the present invention
encompasses kits for use in detecting expression of
PD2 in pancreatic tissues. Such a kit may comprise one
or more pairs of primers for amplifying nucleic acids
corresponding to the PD2 gene. The kit may further

25   comprise samples of total mRNA derived from tissue of
various physiological states, such as normal, early
stage and metastatically progressive tumor, for
example, to be used as controls. The kit may also
comprise buffers, nucleotide bases, and other

30   compositions to be used in hybridization and/or
amplification reactions. Each solution or composition
may be contained in a vial or bottle and all vials
held in close confinement in a box for commercial

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sale. Another embodiment of the present invention
encompasses a kit for use in detecting pancreatic
cancer cells in a biological sample comprising
oligonucleotide probes effective to bind with high

5   affinity to PD2 mRNA in a Northern blot assay and
containers for each of these probes. In a further
embodiment, the invention encompasses a kit for use in
detecting PD2 proteins in pancreatic cancer cells
comprising antibodies specific for PD2 proteins

10   encoded by the PD2 nucleic acids of the present
invention.

15   The following protocols are provided to
facilitate the practice of the present invention.

Cell Lines Utilized in Various Analyses

           The cell line Panc 1 was obtained from American

20   Type Culture Collection (ATCC), and comprises a poorly
differentiated pancreatic adenocarcinoma cell line
(2). The well differentiated pancreatic tumor cell
line HPAF-CD11 was established at Duke University (3).
The sources of other pancreatic cell lines of various

25   states of morphological differentiation were: Colo
357, obtained from George Moore (Denver, Colorado); SW
979, Panc 89, and QGP-1 from H. Kalthoff (Hamburg,
Fed. Rep. of Germany). The following cell lines were
obtained from ATCC: Pancreatic tumor cell lines Hs

30   766T, AsPc-1, BxPc-3, Mia Paca, Capan-1, HGC 25; Human
B lymphocyte cell line NALM; NIH3T3 and the SK-MEL-28
melanoma. Human foreskin fibroblast cells (HUFF) were
obtained from primary cultures established by Dr. Kay

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Singer, Duke University Medical Center. Cell lines
were cultured in Dulbecco's modified Eagle's medium
supplemented with 10% fetal calf serum. Normal
pancreata were obtained from Duke University

5   Comprehensive Cancer Center and the University of
Nebraska Medical Center tumor and tissue banks using
standard procedures.

RNA and DNA purification

10   Total cellular RNA from various tumor cell lines
was isolated by the guanidine isothiocyanate-cesium
chloride cushion ultra centrifugation method (4).
Cells were washed twice with ice cold phosphate
buffered saline and lysed with a solution containing 4

15   M guanidine isothiocyanate, 0.05 M sodium acetate, 250
mM 2-mercaptoethanol. Total RNA was recovered via
sedimentation through a 5.7 M CsCl, 0.025 M sodium
acetate cushion in a Beckman SW 40 Ti rotor
centrifuged at 32,000 rpm for 18 hours. RNA pellets

20   were resuspended in 0.3 M sodium acetate and
precipitated with ethanol. Poly(A+) mRNA was further
purified on two cycles of oligo (dT) cellulose
affinity chromatography. Genomic DNA from HPAF/CD-11
and Panc 1 cell lines was purified by the SDS-

25   Proteinase K digestion method and then extracted with
phenol/chloroform.

Differential Screening and Cloning of PD2 cDNA

The Panc 1 cDNA library was subjected to

30   differential hybridization using single stranded cDNA
probes made from mRNA of Panc 1 and CD-11 cells. The
probes were synthesized using mouse mammary leukemia
virus (MMLV) reverse transcriptase (BRL, Gaithersburg,

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MD) and random hexamer primers (Pharmacia, Piscataway,
NJ). The reaction was carried out in 50 μl of buffer
containing 50 mM Tris pH 8.3, 75 mM KCl, 10 mM DTT, 3
mM MgCl, 3 μg random hexamer primers, 200 μM dATP, 20

5   μM unlabelled dCTP, 200 μM dTTP, 40 μCI α-^{32}P-dCTP, 5
μg actinomycin D, 45 units of RNasin (Promega Biotec,
Madison, WI), and 300 units of MMLV reverse
transcriptase. Following incubation at 37° C for one
hour, the reaction was stopped by adding EDTA to 20

10   mM, and fragments larger than 100 bp were separated by
Sephadex G-100 chromatography. RNA in the cDNA-RNA
hybrid was hydrolyzed at 65°C for 30 minutes in an
equal volume of 0.6 N NaOH and 30 mM EDTA. The
specific activity of cDNA obtained was 0.5 to 1.5 x 10^8

15   cpm/μg of RNA. For screening, triplicate
nitrocellulose membranes were lifted and subjected to
alkaline hydrolysis and neutralization.
Prehybridization, hybridization and washing were as
previously described (5-7). Plaques which hybridized

20   strongly with the Panc 1 cDNA probe, but not with the
HPAF/CD-11 cDNA probe, were selected. The
differential reactivity was confirmed through at least
two additional screening cycles.

        Using a DNA insert derived from a differentially

25   expressed cDNA clone, five additional cDNA clones were
isolated from a normal human fetal pancreatic cDNA
library.

Sequencing Analysis of PD2 cDNA

30   Single phage plaques selected after differential
screening were grown to large quantities using either
plate lysates or liquid culture followed by glycerol
gradient purification (5). *EcoRI* inserts from

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purified DNA were subcloned into pBluescript +/-
vectors (pBS) (Stratagene, La Jolla, CA). Both single
stranded and double stranded templates were sequenced.
Single stranded cDNA was prepared using standard
5   techniques with the + and - strand pBS phagemids that
are hybrid for f1 phage and pBS (Stratagene).
Sequencing was performed by specific primer extension
using Sequenase T4 DNA polymerase under conditions
recommended by the supplier (U.S. Biochemicals,
10   Cleveland, OH). The entire cDNA was sequenced twice,
in both directions.

In vitro transcription and translation of PD2 cDNA

        PD2 cDNA was subcloned into pBS (Stratagene) in
15   both orientations at the EcoRI site. The recombinant
plasmid was linearized with HindIII, extracted with
phenol: chloroform and ethanol precipitated. Linear
plasmids containing inserts in both orientations were
transcribed using T7 polymerase following the
20   instructions of the supplier (Promega). The
transcripts were translated in a rabbit reticulocyte
lysate (Promega) using the manufacturer's procedure
with 50μCi of ^{35}S-methionine (Amersham). The products
were separated by electrophoresis on either 7.5% or
25   10% SDS polyacrylamide gels, and visualized by
autoradiography. Fluorography was used to enhance the
radioactive signal using EN3'HANCE (DuPont-NEN).

Northern and Southern Blot Analysis

30   Total RNA (20 μg) and/or purified poly (A) RNA
were fractionated by electrophoresis on 1.2% agarose
gels containing 0.66 M formaldehyde and transferred to
nitrocellulose via capillary blotting. Genomic DNA

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was digested with the indicated restriction enzymes
and separated on 0.8% agarose gel electrophoresis.
Southern blotting was performed using standard
procedures (5-7). cDNA probes were labeled with ^{32}P

5   dCTP using a random-primed labeling kit (Boehringer
Mannheim, Indianapolis, IN), and were separated from
free label by Sephadex G-50 column chromatography
(Pharmacia). Prehybridization and hybridization for
both Northern and Southern blots were carried out in a

10   solution of 5 X SSPE, 50% formamide, 5 X Denhardt's
reagent, 200 μg/ml of sheared salmon sperm DNA and a
minimum of 10^6 cpm/ml of probe at 42°C for 18 hours.
Blots were washed twice with 2 X SSC containing 0.1%
SDS at room temperature for 15 minutes followed by 4

15   washes with 0.2 X SSC, 0.1% SDS at 60°C.

Sequence Analysis and Molecular Modeling

        Primary cDNA and deduced amino acid sequence
information was analyzed for homology with previously

20   described molecules using the GCG software analysis
program (version 7, Genetics Computer Group, Madison,
WI), and MacVector 3.5 (International Biotechnologies,
Incorporated, New Haven, CT). FASTA searches for
similarity were performed using the following

25   databases: GENBANK (75.0), EMBL (33.0) and Swissprot
(24.0).

        The Molecular Modeling Core Facility at the
Eppley Institute, UNMC, was used for visualizing the
crystallographic structure of proteins with homology

30   to PD2. These included coordinates for aspartate
amino-transferase (1AAT, Protein Data Bank, Brookhaven
National Laboratory) and glutaminyl tRNA synthetase
bound to its cognate tRNA (1GSG, Protein Data Bank,

Brookhaven National Laboratory). The structures were
visualized on a Silicon Graphics work station using
Sybyl software, developed by Tripos Associates, St.
Louis, MO.

5

Chromosomal Mapping

        The chromosomal localization of the PD2 gene was
performed using the gene-based sequence-tagged-site
(STS) mapping method as published earlier (8). The

10   3'-untranslated region (UT) of the PD2 cDNA sequence
was used to design primers for PCR screens of both
CEPH megabase-insert YAC DNA pools, obtained from
Research Genetics, Huntsville, AL. (9), and Coriell
human x rodent somatic cell hybrid DNA pools (10).

15   For PCR amplification, 4 μl of YAC pool DNA was used
in a 15 μl reaction with 250 μm each dNTP, 100 ng of
each primer and 0.4 U of AmpliTaq polymerase in
GeneAmp reaction buffer. Reactions were cycled in a
Perkin Elmer Gene Amp PCR system 9600 as follows: 4

20   minutes at 94°C; then 35 cycles of 15 seconds at 94°C,
1 minute 15 seconds at 55°C, 1 minute 15 seconds at
72°C followed by an extension step of 10 minutes at
72°C. The same set of primers and conditions were
used to screen the somatic cell hybrid panel DNA,

25   NIGMS #1, from Coriell Institute for Medical Research
(Camden, NJ).

Transfection of NIH3T3 Cells with PD2 Sense and
Antisense Constructs

30   The PD2 cDNA was cloned at the EcoRI site of the
pcDNA3.1 vector in the sense and antisense
orientation. The recombinant constructs were
transfected into NIH3T3 cells using the Lipofectamine

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method (GIBCO, BRL).

     The examples presented below are provided to
illustrate certain embodiments of the invention. They
5   are not intended to limit the invention in any way.

                            EXAMPLE I
          Isolation and Sequencing of a cDNA Differentially
               Expressed in Pancreatic Adenocarcinomas
10   Isolation of cDNA
          A cDNA library from a poorly differentiated human
pancreatic tumor cell line, Panc 1, was screened for
differentially expressed mRNAs using single stranded
cDNA probes synthesized from mRNA from the well-
15   differentiated HPAF-CD11 (3) and poorly differentiated
Panc 1 (2) human pancreatic tumor cell lines.
Seventeen clones were obtained that hybridized very
strongly to the Panc 1 probe and did not hybridize to
the HPAF/CD-11 probe. The characterization of two of
20   these clones has previously been published: one
encoded the human ribosomal protein S16 (2); the other
(named PD-1) encoded the human ribosomal protein rpL17
(6). The cDNA reported here is named pancreatic
differentiation 2 (PD2). A representative Northern
25   blot is shown in Figure 1A in which the PD2 cDNA
insert hybridized to a 1.9 kb mRNA transcript. A
comparison of PD2 mRNA levels in these cell lines
reveals that PD2 is expressed at 30-fold higher levels
in Panc 1 when compared to HPAF/CD-11 as determined by
30   densitometric analysis. The same filter was probed
with a human β-actin cDNA as a control for the quality
and quantity of mRNA (Figure 1B). Five additional PD2
cDNA clones were obtained from a normal fetal

pancreatic library.

Sequence of the PD2 cDNA

All cDNA clones isolated from the Panc 1 cDNA
library and from the normal fetal pancreatic cDNA
5   library showed 100% identity in sequence. The
complete nucleotide sequence and the deduced amino
acid sequence of the longest PD2 cDNA (1.9kb) are
shown in Figure 2. The cDNA sequence contained a 5'
untranslated region of 156 base pairs and a 3'
10   untranslated region of 138 bp. The non-coding 3'
region contained the polyadenylation signal AATAAA as
underlined in Figure 2. An open reading frame from
nucleotide 157 to nucleotide 1752 yields a predicted
translation product of approximately 60 kDa. A search
15   of the GENBANK and EMBL databases revealed that PD2 is
a newly identified sequence, as the nucleotide
sequence of PD2 did not show identity to any other
deposited sequence.

Characterization of the PD2 cDNA

20   The PD2 mRNA transcript was expressed using T7
RNA polymerase and translated in rabbit reticulocyte
lysate in the presence of ^{35}S methionine. The *in vitro*
translation products were analyzed by 7.5% SDS-PAGE
and the results are shown in Figure 3. When PD2 cDNA
25   was placed in the correct orientation, a protein of
approximately 70 kDa was also seen along with three
other protein bands of about 43 kDa, 44 kDa, and 45
kDa (lane 1). This pattern of reactivity was seen
when non-reducing SDS-PAGE gels were run on these
30   samples (data not shown). Similar products were not
produced in lysates that contained linear pBS DNA cut
with Hind III (lane 3), full length PD2 in an
antisense orientation, (lane 2) or lysate alone (lane

4). A positive control for *in vitro* transcription/
translation analysis using the unrelated cDNA clone
PD-1 (6) showed translation of a protein with the
expected size of 17 kDa. The discrepancy between the

5   observed migration of PD2 (70 kDa) and several smaller
species and the calculated mass (60 kDa) may be due to
posttranslational modification in the reticulocyte
lysate or intrinsic properties of the protein. It is
unlikely that the different protein forms are

10   consequences of mutations in the insert that alter the
reading frame, or contaminated plasmid preparations,
since three independent preparations from a single
colony gave essentially the same pattern (data not
shown).

15   **Chromosomal Localization of the PD2 Gene**
The PD2 gene was mapped to the short arm of
chromosome 19 (19p13.11-q11). This location was
obtained by linking the YAC that PD2 mapped to (CEPH
785_d_8) out four levels to another YAC (CEPH

20   968_g_5). This YAC then had an sequence-tagged-site
(STS) (D19S215) mapped to it, which is mapped to
Genethon position 0.38 on Chromosome 19. This
position could not be translated to the cytogenetic
map because a map for chromosome 19 is not yet

25   available for this region. However, the STS (D19S215)
has been mapped on the Lawrence Livermore National
Laboratories physical map of chromosome 19 about 20Mb
from the p-ter. Using the sizes of the four YACs that
link PD2 to this STS, a region of 19p13.11-q11 was

30   determined.

EXAMPLE II

Characterization of the PD2 Protein

Functional Motifs in the PD2 Protein

A search for protein motif similarities in the
GENBANK, EMBL, PDB and Swiss Prot databases, using BLAST
and the FASTA program of the GCG sequence analysis software
package, revealed that the PD2 protein has some regions of
similarity to several known proteins (Table I).

Table I: Proteins with partial homology to PD2

PROTEIN	ACCESSION NUMBER*	POSITION AND PERCENTAGE IDENTITY
Yeast transcriptional factor PAFI	P38351	277-336, 26% in 60 residues
INCENP nuclear protein	P53352	103-192, 23% in 90 residues
Glucokinase	pdb/1glk/80 9431	243-280, 36% in 38 residues
E.coli valyl tRNA synthetase	P07118	36-133, 25% in 98 residues
RNA polymerase sigma 54 factor	p2469	68-100, 24% in 33 residues
Yeast ATP dependent RNA helicase	P15424	145-159, 47% in 15 residues
Yeast Myosin-like protein MLPI	Q02455	59-93, 37% in 35 residues
E.coli Aspartate Aminotransferase	P14909	200-270, 27% in 71 residues
Simian immunodeficiency virus reverse transcriptase	P5896, P5897, P19509 P112502	295-326, 38% in 32 residues 318-364, 24% in 47 residues
Murine leukemia virus reverse transcriptase	P11227, P03355, P03357	236-271, 27% in 37 residues
Recombinase flp protein	P13784	300-339, 23% in 40 residues
Trypanosome RNA polymerase	P16355	313-366, 19% in 54 residues
cAMP dependent protein kinase, type 1 regulatory protein	PP12849	263-345, 23% in 83 residues
Modification methylase RSR1	P14571	312-363, 27% in 52 residues
Maternal effect protein oscar (Drosophila)	P25158	117-176, 26% in 63 residues
Inclusion body matrix protein (Viroplasmin)	P09524	125-184, 20% in 67 residues

48

*   Accession numbers are from Swissprot (P and Q), PDB, and
        EMBL databases.   References of each of the sequence is
        available in the databases.

5

Some of the regions with identity coincide with

10   functionally important regions of proteins, including

a yeast transcriptional factor (11); INCENP nuclear

protein (12); Glucokinase (13); valyl-tRNA synthetase

(14); aspartate amino transferase (15); the cAMP

binding domain of the bacterial Catabolite Activator

15   Protein (16); and eukaryotic regulatory type I subunit

of a cAMP-dependent protein kinase (17). There is

also similarity with certain functionally important

regions of several other proteins with known

biological activity that are shown in Table I.

20   The PD2 protein depicted in Figures 4A and 4B

contains the following motifs, a putative nuclear

localization signal (KKRK) at residues 269 - 272 (18);

a putative arginine rich RNA binding domain

(RVRLSKRRAKA; SEQ ID NO: 14) at residues 329 - 339

25   with homology to a consensus sequence described in

reference (19); and a putative helix-loop helix domain

near the amino terminus with homology to a family of

helix-loop-helix (HLH) proteins described previously

(20) that include members of the myc and myo D

30   families, and several HLH proteins that play important

roles in Drosophila development. Figure 5 shows the

alignment of HLH domain of PD2 with the HLH domain in

Drosophila hairy protein, a negatively acting member

of this family that regulates expression of other

35   genes involved in segmentation and bristle pattern

development (21). Some members of this family of

proteins contain a basic region important for DNA

binding which precedes the first helix. The HLH

49

domain itself has been shown to be required for
heterodimerization of these family members with other
proteins. Some negatively acting HLH proteins that
associate with and block the action of positively
5   acting HLH proteins lack the basic region preceding
the HLH domain, or contain prolines in their basic
region (20). The PD2 protein is similar to the latter
of these, in that it contains prolines and several
basic residues in the region immediately preceding the
10   putative HLH domain (residues 11-24). See Figure 5.

Another region of PD2 (residues 263 - 345, 23% in
83 residues, Figure 4) demonstrated significant
homology with the type I regulatory chain of an
intracellular cAMP dependent protein kinase (residues
15   265 -343,) (17). The region of homology fell within
one of the two cAMP binding domains of the regulatory
chain of this enzyme. Furthermore, the PD2 amino acid
sequence showed significant homology with the
conserved residues found in the cAMP binding site of
20   the *E. coli* catabolite activator protein (16) and both
of the cAMP binding sites found in the regulatory
chain of the eukaryotic protein kinase, that included
critical glutamic acid and arginine residues known to
be important in forming hydrogen bonds involved in
25   binding the cAMP and forming the pocket around it
(16). See Figure 6. The biological relevance of this
region of homology with the cAMP binding element is
further supported by the fact that an overlapping
sequence (described below) showed homology with an
30   arginine rich motif found in several potential RNA
binding proteins (19). PD2 also contained several
phosphorylation sites similar to histone kinase and
casein kinase.

PD2 Prot in in the Cell

To study the production of PD2 protein and examine its localization within the cell, polyclonal antibodies were generated against two synthetic
5   peptides of the deduced amino acid sequence of PD2. The peptides were selected based on their high antigenic index as analyzed by the MacVector Program. PD2 Peptide 1 (PD2p1) corresponds to amino acids 142-162 and has the following sequence:
10   NH_2-RYGISNEKPEVKIGVSVKQQF-COOH   (SEQ ID NO:10)
PD2 peptide (PD2p2) corresponds to amino acids 327-348 and has the following sequence:
NH_2-ETRVRLSKRRAKAGVQSGTNAL-COOH   (SEQ ID NO:11)

The peptides listed above were conjugated to KLH
15   and the resulting immunogen used to immunize rabbits. Serum was then isolated from a rabbit immunized with PD2p2 peptide and PD2 antibodies purified using peptide affinity chromatography. The antibodies were then used as probes for Western blotting of
20   cytoplasmic and nuclear extracts from HPAF/CD11 and Panc1 cells. The PD2p2 antibody reacted with a protein of approximately 70 kd in nuclear extracts from both cell lines. The intensity of the PD2 protein band in the nuclear extract was 30 fold higher
25   in Panc1 cells as compared to HPAF/CD11 cells. See Figure 7. A similar level of over expression was seen in PD2 mRNA and gene copy number in the Panc1 line.

30

EXAMPLE III

Role of PD2 in Differentiation and Tumor Progression

Differ ntial Expression of PD2

Expression of the PD2 gene was evaluated further
in a panel of 14 pancreatic tumor cell lines
representing various morphological stages of
differentiation. The putative differentiation grade
for the various tumor cell lines was determined from
the published morphological and ultrastructural
descriptions of the cell lines and corresponding
tumors (22). Total RNA from these tumor cell lines
were fractionated, Northern blotted and probed with
PD2 cDNA. The results of this experiment are shown in
Figure 8A. The PD2 cDNA probe hybridized to a message
size of approximately 1.8 kb in all cell lines, but
its expression was significantly elevated only in Panc
1. Fig 8B shows rehybridization of the same filter
with a β-actin probe confirming equal loading of the
gel.

Expression of PD2 mRNA was also evaluated in
colon and breast tumor cell lines, human foreskin
fibroblasts, a Human B lymphocyte cell line and normal
pancreatic tissues. The results, shown in Figure 9A,
revealed relatively low levels of mRNA transcripts for
PD2 in all cell lines examined except Panc 1. Figure
9B is a control blot showing equal loading of RNA.

Differential Amplification of the PD2 Gene

Purified genomic DNA from the poorly
differentiated cell line Panc 1 and well
differentiated cell line HPAF/CD 11 were digested with
EcoRl, BamHl and Hindlll, fractionated by agarose gel
electrophoresis, Southern blotted and hybridized to

the PD2 cDNA probe as shown in Figure 10. The probe
hybridized to two or more fragments raising several
possibilities. PD2 could be part of multi-gene
family, there may be pseudogenes for PD2, or PD2 is a
5   part of large gene that contains multiple restriction
sites. One of the bands showed a 30-fold
amplification in Panc 1 DNA as compared to HPAF/CD11,
suggesting that this corresponds to the gene encoding
the transcribed product seen in Panc 1 cells.
10   Furthermore, Southern blot analysis of EcoR1-digested
DNA from a large panel of tumor cell lines including
six pancreatic tumor cell lines, three breast tumor
cell lines and three colon tumor cell lines confirmed
that amplification of the PD2 gene occurred only in
15   the Panc 1 cell line as shown in the Figure 11.

Tumor specimens were assessed for the
amplification of of the PD2 gene as shown in Figure
12. One tumor biopsy sample also showed amplification
of the PD2 gene. The same blot was stripped and
20   reprobed to confirm equal loading of the lanes. These
results demonstrate that PD2 is involved in the
development of pancreatic cancer.

The expression of the PD2 gene was also assessed
in normal human adult and fetal pancreata. The
25   results are shown in Figure 13. Normal adult pancreas
(ages 30-59) showed very low to undetectable levels of
PD2 expression. However PD2 expression was
consistently observed in all fetal tissues examined
(age 18-24 weeks). Expression of the PD2 gene in
30   fetal but not in adult pancreas further supports a
role for PD2 in transformation and differentiation of
pancreatic cells.

EXAMPLE IV

Expression Construct Analysis of PD2

Construction of FLAG Epitope-Tagged PD2

A cDNA molecule was prepared which generated a
fusion protein comprising PD2 and the Flag epitope.
For this purpose, a double-stranded, synthetic
oligonucleotide was designed to encode DYKDDDGSKSAIF
which was inserted into the unique BglII site (bp
1511) of the PD2 cDNA. The in-frame insertion of the
oligonucleotide was verified by sequence analysis.

Figure 13 shows the results of Western blot
analysis confirming expression and translation of the
cDNA fusion construct. The results showed that
epitope tagged PD2 protein is localized mainly in
nuclear lysates.

In additional experiments, the 1.9 kb PD2 cDNA
was placed under control of a strong CMV promoter by
using the pCDNA3.1 vector for driving expression of
PD2 in NIH3T3 cells. Stable transfectants were
selected on geneticin (G418). Expression of PD2-
specific mRNA in the NIH3T3 cells transfected with PD2
is shown in Figure 16. There was no detectable mRNA
for PD2 in the anti-sense PD2 transfected NIH3T3
cells, designated herein as PD2ASNIH3T3. NIH3T3 cells
transfected with a sense construct, designated herein
as PD2SNIH3T3, showed the expression of appropriate
size mRNA for the PD2 gene.

Determination of growth kinetics revealed a
shorter population doubling time for PD2SNIH3T3 cells
as compared to PD2ASNIH3T3 cells or control,
untransfected NIH3T3 cells. Viable transfected cells
(5 x 10³) were plated in triplicate in RPMI medium with
10% fetal calf serum (Day 0). The following day the

medium was changed and cells were harvested in 0.125%
trypsin-0.02% EDTA every day and counted. Figure 17
shows the mean values of three dishes over an 8 day
time course. The data reveal that NIH3T3 cells

5   expressing PD2 in the sense orientation divide more
rapidly than control cells not expressing PD2 or cells
expressing PD2 in an antisense orientation.

To investigate transformation properties of
PD2SNIH3T3 cells in vivo, BALB/c, nu/nu were

10   inoculated subcutaneously with PD2SNIH3T3, PD2ASNIH3T3
and control NIH3T3 cells. Groups of five mice were
used and challenged with 1×10^7 cells of each cell
type. Mice were palpated biweekly over a period of 5
weeks. The time to tumor formation(LP), the number of

15   mice developing tumors, the doubling time of the
tumors, and the size of the tumors were noted. Tumor
volumes were calculated using width (a) and length (b)
measurements ($a^2 \times b/2$, where a<b). PD2SNIH3T3 cells
formed tumors in all mice after a latent period of 7

20   days, whereas no tumores developed in mice receiving
identical doses of PD2ASNIH3T3 or NIH3T3 cells over an
observation period of five weeks. See Figure 18.

These results demonstrate that the PD2 gene can
transform NIH3T3 cells and disturb the components that

25   regulate growth rate in transfected cells.

EXAMPLE V
GENETIC TESTING OF TUMOR BIOPSIES FOR THE PRESENCE OF PD2

30

As described in the previous examples, PD2 is
overexpressed in pancreatic adenocarcinoma. The
availability of the nucleic acids having the sequence

of Sequence I.D. Nos. 1 as well as the primer sets set
forth below provide reagents for genetic testing in
patients for the presence or absence of amplified PD2.

5   PD2 forward primer:

5' TTCAGTCAGGCACCAACG 3' (SEQ ID NO:12)

PD2 reverse primer:

5' CGCTGGCCACCCCCATTG 3' (SEQ ID NO:13)

        DNA may be isolated from biopsy samples. Such

10   procedures are known to those of skill in the art.
The DNA is suspended in 600 microliters of 50 mM NaOH
in a 1.5 ml eppendorf tube. The tube is then vortexed
for 10 seconds followed by a 5 minute incubation in a
95 degree hot water bath. Following this incubation,

15   60 microliters of Tris (1mM, pH 8.0) is added to the
tube and the sample vortexed for an additional 10
seconds. The tube is centrifuged in a microfuge for 1
minute to pellet the DNA. The supernatant is then
discarded and the sample frozen or the DNA processed

20   for PCR. Following DNA amplification, the DNA is
sequenced in an automated DNA sequencer to confirm the
results obtained with PCR.

25

                        EXAMPLE VI

        GENERATION OF A HAMSTER MODEL FOR PANCREATIC
                        CARCINOGENEIS

30   Little is known about the etiology, pathogenesis
and molecular basis of pancreatic cancer (PC).
Deletions, mutations, and rearrangements normally
activate proto-oncogenes and inactivate anti-oncogenes

(tumor-suppressor genes). These genetic alterations
culminate in molecular events leading to deregulation
of cell proliferation. Recent studies have shown that
a preferred subset of normal genes is altered in human

5   PC biopsies. Although alterations of these genes have
been found in established tumors, nothing is
known about early genetic events at the initiation
stage of pancreatic carcinogenesis. Moreover, it is
equally unclear whether alteration of one gene

10   is sufficient or whether several simultaneous or a
chain of multiple genetic abnormalities is required
for the initiation of the uncontrolled growth.

        It is well established that in the hamster
pancreatic cancer model, which mimics the human

15   disease in many clinical and biological aspects,
cancer develops not only from ductal/ductular cells
but also from within islets, most probably from stem
cells. The hormonal abnormalities found in more than
80% of pancreatic cancer patients, including

20   development of diabetes and increased levels of
insulin and islet amyloid polypeptide, ubiquitously
reflect the involvement of the endocrine pancreas in
the pathogenesis of the pancreatic cancer.

        We have recently established an in vitro hamster

25   islet culture, in which the stepwise cytological,
immunohistochemical, cytogenetic and molecular
biological changes could be followed. We have shown
that these hamster pancreatic islet cells can be
transformed in vitro by the pancreatic carcinogen,

30   N-nitrosobis (2-oxopropyl)amine (BOP). After four
weeks of treatment with BOP, the cultured islet cells
showed accelerated growth and pleomorphism.
Anchorage independent and in vivo growth was not seen

before week 19 of treatment. The mutation of the K-ras gene at codon 12 (GGT6GAT) was found at this stage. This abnormality is consistent with findings in over 90% of human and BOP-induced pancreatic cancer. In

5   vivo, transformed hamster islet cells formed a poorly differentiated invasive cancer. Cytogenetic analysis of transformed hamster cells revealed deletions of chromosome Y and loss of heterozygosity of chromosomes 7 and 11. Hence, this system presents a unique

10 model for elucidating stepwise early genetic alterations during pancreatic carcinogenesis.

Inasmuch as BOP triggers loss or inactivation of tumor suppressor genes, activation of oncogenes, and/or differential expression of

15 tumor-associated genes in normal pancreatic cells at the initiation of carcinogenesis. The genetic alterations caused by these carcinogens appear to be critical in the development of pancreatic cancer.

This hamster model may be manipulated to assess

20 the role of PD2 expression in the malignant transformation of the pancreas. Altered PD2 encoding nucleic acids may be introduced into these cells and effects of cell growth rates and morphology assessed. Additionally, the PD2 expressing cells may be treated

25 with test compounds to identify those compounds which reverse the malignant phenotype. Such compounds may have beneficial therapeutic value in the treatment of pancreatic carcinomas.

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     While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various

10 modifications may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.

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